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**The Flavor and Fragrance High Production Volume
Consortia**

The Terpene Consortium

Robust Summaries for Estragole

Estragole

CAS No. 140-67-0

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FFHPVC Terpene Consortium Registration Number

**Submitted to the EPA under the HPV Challenge Program by:
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The Flavor and Fragrance High Production Volume Consortia

Robust Summaries for Estragole

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al.*, 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 Melting Point

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Comparison of experimental melting points for a group of structurally related allylalkoxybenzene and propenylalkoxybenzene derivatives
GLP	No
Melting Point	1 °C
Comment on method	The incremental change in experimental melting point between 4-methoxy- (21 °C) and 3,4 dimethoxy-1-propenylbenzene (16-17 °C) is 4-5 °C. Based on these data and the fact that 3,4-dimethoxy-2-propenylbenzene exhibits a melting point of -4°C

	(Chemical Rubber Handbook), the melting point of 4-methoxy-2-propenylbenzene (estragole) is estimated to be 1 °C
Data Qualities Reliabilities	Reliability code 2. Iteration of melting point data based on known changes in chemical structure is reliable provided data is available for a sufficient number of congeners..
References	Chemical Rubber handbook, 2004.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated/Mean or weighted (adapted Stein and Brown method)
GLP	No
Melting Point	-1.19 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

1.2 Boiling Point

Substance Name	Estragole
CAS No.	140-67-0
GLP	Ambiguous
Boiling Point	216 deg C
Pressure	764
Pressure Unit	mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Merck Index (1998) The Merck Index, 12th edition, Merck & Co., Inc. Whitehouse Station, NJ.

Substance Name	Estragole
CAS No.	140-67-0
GLP	Ambiguous

Boiling Point	216 °C
Pressure	760
Pressure Unit	mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Fragrance Materials Association (FMA) Reported values for boiling point of estragole.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Adapted Stein and Brown method
GLP	No
Boiling Point	209.93 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

1.3 Vapor Pressure

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Experimental
GLP	No
Year	1947
Vapor Pressure	1 mm Hg
Temperature	52.6 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.

References

Stull D.R. (1947) Vapor pressure of pure substances. Organic Compounds. Ind Eng Chem., 39, 517-540.

Substance Name	Anethole (isomer unspecified –surrogate for estragole)
CAS No.	104-46-1
Remarks for Substance	Data is for anethole, isomer unspecified
Method/guideline	Measured
GLP	Ambiguous
Vapor Pressure	0.041 mm Hg (5.45 Pa)
Temperature	21 °C (294 K)
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Daubert T.E. and Danner, R.P. (1989) Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Taylor and Francis, Washington, DC.418

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated
Vapor Pressure	0.09 mm Hg (12 Pa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Reported values of vapor pressure for estragole. Unpublished report.

Substance Name	Anethole (isomer unspecified –surrogate for estragole)
CAS No.	104-46-1
Remarks for Substance	Data is for <i>trans</i> -anethole
Method/guideline	Calculated
Vapor Pressure	0.05 mm Hg (6.67 Pa)
Temperature	20 °C

Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Reported values of vapor pressure for trans-anethole. Unpublished report.

1.4 n-Octanol/Water Partition Coefficients

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated
Log Pow	3.47
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency.

1.5 Water Solubility

Substance Name	Estragole
CAS No.	140-67-0
Method/Guideline	Measured
GLP	Ambiguous
Year	1992
Value (mg/L) at Temperature	178 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.

Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	WSKOWIN EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski, S.H. and Dannenfelser, R.M., 1992)

Substance Name	Anethole (isomer unspecified –surrogate for estragole)
CAS No.	104-46-1
Remarks for Substance	Data is for anethole, isomer unspecified
Method/Guideline	Measured
GLP	No
Value (mg/L) at Temperature	111 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: peer reviewed reference
References	WSKOW EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski S.H., and Dannenfelser, R.M., 1992)

Substance Name	Estragole
CAS No.	140-67-0
Method/Guideline	Calculated
Remarks for Test Conditions	Used an estimated log Kow of 3.47
Value (mg/L) at Temperature	84.55 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOWIN EPI Suite (2000b) US Environmental Protection Agency.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 Photodegradation

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated
Test Type	AOPWIN
Half-life t_{1/2}	2.36 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

2.2 Biodegradation

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for 3-methoxy-4-hydroxyallylbenzene
Method	OECD Guideline 301B
Test Type	Sealed vessel test (CO ₂ production test)
Year	1994
Innoculum	10% by volume of secondary effluent from an unacclimatized activated sludge
Remarks for Test Conditions	The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 17-22 °C. The mean percentage biodegradation was calculated from 4 vessels on day 28.
Degradation % After Time	100.4% (98.0-102.8-%)
10 day window criteria	Yes
Total degradation	Yes

Conclusion Remarks	Substances is classified as readily and ultimately biodegradable.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Quest International, Inc. (1994a) The ultimate and readily biodegradation of eugenol. Unpublished report.

Substance Name	Anethole (isomer unspecified –surrogate for estragole)
CAS No.	104-46-1
Remarks for Substance	Data is for <i>p</i> -(2-propenyl)anisole isomer, anethole
Method	OECD Guideline 301B
Test Type	Sealed vessel test (CO ₂ production test)
Year	1994
Innoculum	10% by volume of secondary effluent from an unacclimatized activated sludge
Remarks for Test Conditions	The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 20-24 °C. The mean percentage biodegradation was calculated from 4 vessels on day 28.
Degradation % After Time	91.0% (90.7-91.2%)
10 day window criteria	Yes
Total degradation	Yes
Conclusion Remarks	Anethole is classified as readily and ultimately biodegradable.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Quest International, Inc. (1994b) The ultimate and readily biodegradation of anethole. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Method	Calculated
Test Type	BIOWIN
Results	Probability of rapid biodegradation - linear model 0.8636 - nonlinear 0.9766. Expert survey results - Ultimate survey model: 2.7387 (weeks-months); Primary survey model: 3.6425 (days-weeks)

Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	BIOWIN EPI Suite (2000) U S Environmental Protection Agency (Meylan W., 1994).

Fugacity

Substance	Estragole
CAS	140-67-0
Model Conditions	25 C, 1000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC Fugacity Level III
Input parameters	MW (148.21), VP(0.041 mm Hg), log Kow (3.47), water solubility (111 mg/L), MP (1 °C), BP (216°C)
Year	2000
Media	Air-Water-Soil-Sediment Partition Coefficients
Model data and results	Compartment half-lives, hours: Air=3.92; Water=900;Soil=900;Sediment=3600
Estimated Distribution and Media Concentration	Air=0.391% Water=25.1% Soil=73.4% Sediment=1.12%
Conclusion remarks	Substance is predicted to persist in the environment for 574 hours.
Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. Environmental Toxicology and Chemistry, 15(9), 1627-1637.
Substance Name	Estragole

CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, calc. log Kow, exp. water solubility, calculated MP, exp. BP & VP
Media	Air
Estimated Distribution and Media Concentration	0.556%
Remarks	Half-life = 3.92 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

Substance Name	Estragole
CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Water
Estimated Distribution and Media Concentration	19.7%
Remarks	Half-life = 900 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.

References

Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

Substance Name	Estragole
CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Soil
Estimated Distribution and Media Concentration	78.8%
Remarks	Half-life = 900 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

Substance Name	Estragole
CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Sediment
Estimated Distribution and Media Concentration	0.88%

Remarks	Half-life = 3600 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

3 ECOTOXICITY

3.1 Acute Toxicity to Fish

Substance Name	Methyl eugenol (surrogate for estragole)
CAS No.	93-15-2
Remarks for Substance	Assay: >97%
Test Type	Experimental
GLP	No
Year	1975
Species/Strain/Supplier	Fish/Rainbow trout
Exposure Period	96 hour
Remarks for Test Conditions	Ten fish were used. Each material tested at 5 concentrations. Control groups conducted concurrently. The fish were observed for 96 hours. The fish were placed in bioassay vessels containing reconstituted water, and the test material in acetone was added to the vessels. Ten fish per concentration were used, and each material was tested at five concentrations. Control groups of fish in untreated water and in water to which acetone only was added were observed concurrently. The fish were observed for 96 hours and all deaths and/or behavioral reactions were recorded. The concentration of dissolved oxygen was measured in all solutions in which deaths occurred to be sure the test water contained sufficient oxygen: dissolved oxygen concentrations above 4 mg/liter (4ppm for the warm-water fish (bluegills) or above 5 mg/liter for cold-water fish (rainbow trout) were considered adequate. The median lethal concentrations (LC50) of the test materials were calculated by Litchfield and Wilcoxon method.
Nominal concentrations as mg/L	3.2-10 mg/L
Endpoint value	6 mg/L 95% C.I. (4.9-7.2)
Reference substances (if used)	Toxaphene
Conclusion remarks	The authors concluded that estragole was of a low order of toxicity to fish. 95% confidence level was 4.9-7.2 ppm in rainbow trout. Concentration range was 3.2-10.0 ppm in rainbow trout. With higher doses trout became quiescent and flaccid, swimming or lying on their sides, with slow respiration. Dark discoloration of the integument was also observed.

Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Beroza M., Inscoe M., Schwartz P., Kepliknger M. and Mastri C. (1975) Toxicology and Applied Pharmacology 31, 421-429.

Substance Name	Methyl eugenol (surrogate for estragole)
CAS No.	93-15-2
Remarks for Substance	Assay: >97%
Test Type	Experimental
GLP	No
Year	1975
Species/Strain/Supplier	Fish/Bluegill sunfish
Exposure Period	96 hour
Remarks for Test Conditions	Ten fish were used. Each material tested at 5 concentrations. Control groups conducted concurrently. The fish were observed for 96 hours. The fish were placed in bioassay vessels containing reconstituted water, and the test material in acetone was added to the vessels. Ten fish per concentration were used, and each material was tested at five concentrations. Control groups of fish in untreated water and in water to which acetone only was added were observed concurrently. The fish were observed for 96 hours and all deaths and/or behavioral reactions were recorded. The concentration of dissolved oxygen was measured in all solutions in which deaths occurred to be sure the test water contained sufficient oxygen: dissolved oxygen concentrations above 4 mg/liter (4ppm for the warm-water fish (bluegills) or above 5 mg/liter for cold-water fish (rainbow trout) were considered adequate. The median lethal concentrations (LC50) of the test materials were calculated by Litchfield and Wilcoxon method.
Nominal concentrations as mg/L	3.2-10 mg/L
Endpoint value	8.1 mg/L 95% C.I. (7.4-9.0)
Reference substances (if used)	Toxaphene
Conclusion remarks	The authors concluded that estragole was of a low order of toxicity to fish. Calculated LC50 95% confidence limits were 7.4-9.0 ppm in bluegill sunfish. Concentration range was 3.2-10.0 ppm. With higher doses Bluegill sunfish became quiescent and flaccid, swimming or lying on their sides, with slow respiration. Clinical signs at 6 mg/L and greater.

Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Beroza M., Inscoc M., Schwartz P., Kepliknger M. and Mastri C. (1975) Toxicology and Applied Pharmacology 31, 421-429.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hours
Remarks for Test Conditions	Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 °C.
Endpoint value	LC50 = 4.561 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U S Environmental Protection Agency.

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Chemical Assay: 98.9%
Method/guideline	OECD 202
Test Type	Experimental
GLP	Yes
Year	2003
Analytical procedures	HPLC/UV detector
Species/Strain	Daphnia magna/Aquatic Biosystems, Inc.
Test details	48 hrs.
Remarks for Test Conditions	Juvenile daphnids (<24 hours old) (10/group) produced from an in-house culture of adults were maintained at the contract laboratory under test conditions for 45 days. During the 48 hours prior to testing, the daphnid culture was maintained in 100% dilution water under static, renewal conditions for 48 hours. There was no mortality during the 48 hours prior to test and the test organisms appeared free of disease, injuries, or abnormalities. The daphnid culture produced young before day 12 and a subsample of adults produced on average, more than 3 young per day during the 7 days prior to the beginning of the test. The test substance was provided via an intermittent flow proportional diluter. Ten daphnid were randomly selected for each replicate test. Tests were performed at 5 nominal concentrations. During the 48-hr test, daphnid were exposed to 16 hours of light and 8 hours of darkness. Mortality, immobility, and sub-lethal effects were determined visually at 0, 24, and 48 hours. Test temperature was maintained at 19.5-20.7 °C
Nominal concentrations as mg/L	0, 6.2, 10, 18, 29, 48, and 80 mg/L
Measured concentrations as mg/L	0, 4.73, 7.58, 12.9, 34.5, and 56.9 mg/L
Unit	mg/L
EC50, EL50, LC0, at 24,48 hours	48-hr EC50=2.65 mg/L and 48 hr LC50=3.11 mg/L; NOEC 1.14 mg/L
Biological observations	The number of surviving daphnids at 48 hours for duplicate runs at each mean measured concentration was: 0 mg/L, 9/9; 4.74 mg/L, 10/10 & 10/10; 7.58 mg/L, 9/10 & 10/10; 12.9 mg/L, 1/10 & 2/10; 20.4, 34.5, and 56.9 mg/L, 0/10 & 0/10.
Control response	yes

satisfactory?

Appropriate statistical evaluations?	Probit method (Stephan, 1978)
Remarks fields for results	The measured concentrations after 24 and 48 hours were 70-76% of the nominal concentrations, with the concentration being held steady throughout the test period. The respective ranges for conductivity, pH, dissolved oxygen, and temperature were: 550 umhos/cm, 7.3-7.5, 7.8-8.9 mg/L, and 19.5-20.7C, respectively.
Conclusion remarks	The acute 48-hour EC50 and LC50 for estragole in <i>Daphnia magna</i> under semi-static conditions were 8.87 and 10.5 mg/L, respectively. The NOEC for estragole in <i>Daphnia magna</i> is 4.73 mg/L
Reliabilities	Reliability Code No. 1. Reliable without restriction.
Remarks for Data Reliability	The data are obtained by a recognized guideline method and are consistent with chemical structure.
References	Ward T. (2003) Acute toxicity test with estragole and the <i>Daphnia magna</i> . Study No. 2504-FF. Private communication to FFHPVC. Unpublished Report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Test substance was estragon oil (tarragon oil). Typical composition of estragon oil is (70-88% estragole).
Method/guideline	OECD Guideline 202-I
Test Type	Experimental
GLP	Yes
Year	2001
Species/Strain/Supplier	<i>Daphnia magna</i> /Straus
Test Details	48 hours
Remarks for Test Conditions	Groups of 20 <i>Daphnia magna</i> (Karlsruhe, GDR)(5/1ml test volume) were exposed to test concentrations of 0, 0 (acetone solvent), 3.8, 7.5, 15.0, 30.0, or 60.0 mg/L of estragon oil for 48 hours. Solution temperature and pH were maintained at 20-20.5 C and 7.98. Invertebrates were held for 16 hours in daylight followed by 8 hours of dark. The conductivity of the water was 0.4 to 1.5 uS/cm and water hardness was 200 mg/L. <i>Daphnia</i> (2-24 hrs. old) were decanted into 25 ml glass beakers, each containing 10 ml of test solution with the test substance in various concentrations. Test solutions were prepared by emulsification of the test substance in water with acetone. There were 5 <i>Daphnia</i> per beaker and 5 beakers per each concentration. Test conditions consisted of a 16 hr./8 hr. light/dark cycle, a light intensity of 200 lx, oxygen concentration

	of 8.3-8.6 and temperature of 20.0-20.5 degrees Celsius. The Daphnia are examined for mobility after 24 and 48 hours. Daphnia which showed no reaction after 15 seconds were considered immobile. The pH, oxygen content and temperature were measured at the beginning and end of the test. Probit analysis was performed to determine the EC50.
Nominal concentrations as mg/L	0,3.8,7.5,15.0, 30.0, or 60.0
Unit	mg/L
EC50, EL50, LC0, at 24,48 hours	EC50 = 30.5mg/l (95% CI, 13.3-48 mg/L)
Biological observations	<p>No reduction in swimming mobility was observed at 0, 3.8, 7.5 or 15 mg/L at 3, 24, or 48 hours. At 30.0 mg/L reduction in swimming mobility was reported for 5/20, 5/20, 8/20 at 3, 24, or 48 hours, respectively.</p> <p>3.8, 7.5 and 15 mg/L- No effect on swimming capacity after 48 hours</p> <p>30.0 mg/L- Statistically significantly reduction in the swimming capacity was observed.</p> <p>60 mg/L- 100% reduction in swimming capacity after 48 hours</p>
Control response satisfactory?	Yes
Appropriate statistical evaluations?	Probit Analysis
Remarks fields for results	Measurement of pH, Oxygen concentration, and temperature at 0 and 48 hours revealed no significant change (7.69-8.02) in pH, O2 concentration (8.3-8.6), or temperature (20 to 20.3C)
Conclusion remarks	The EC50 for <i>Daphnia magna</i> in a static immobilization study was 30.5 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Data Reliability Remarks	Code 1. Guideline study.
Reference	Barth M. and Winkler, J (2001) Testing for acute toxicity of estragon oil (<i>Artemisia dracuncululus</i> L.) in Daphne - <i>Daphnia magna</i> . Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	ECOSAR
Test Type	Calculated

Species/Strain/Supplier	<i>Daphnia magna</i>
Test Details	48 hours
Remarks for Test Conditions	Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 C.
Unit	mg/L
EC50, EL50, LC0, at 24,48 hours	LC50 = 5.410 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U S Environmental Protection Agency.

3.3 Acute Toxicity to Aquatic Plants

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Assay: 98.9%
Method/guideline	OECD 201 Guideline
Test Type	Experimental
GLP	Yes
Year	2003
Species/Strain/Supplier	Green algae/Selenastrum capricornutum/UTEX 1648
Exposure period (duration)	72 hrs
Analytical monitoring	HPLC/UV detector
Remarks for Test Conditions	Green Algae/Selenastrum capricornutum/U. of Texas was maintained at test conditions for 14 days prior to the test. The culture was growing in at least 2 subcultures prior to the initiation of the test. In a range finding test, the number of cells/mL was 76% of controls at 0.01 mg/L, >100% of controls at 0.10 mg/L, 51% at 1.0 mg/L, and <3% at 10 and 100 mg/L after three days. In the definitive test, algae was treated with nominal concentrations of 0, 0.13, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/L for 72 hours. pH was adjusted to 7.5 and solutions were exposed for 24 hours of light of intensity, 400 foot candles. The number of algal cells/mL as well as relative size, cell shapes, color, adherence and aggregation of cells was determined. At 24, 48, and 72 hours 3 treatment and 6 control vessels were sacrificed to determine the number of algal cells/mL.

Concentrations were determined by HPLC.

Nominal concentrations as mg/L	0, 0.13, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/L
Measured concentrations as mg/L	Initial mean measured concentrations 0, 0.118, 0.223, 0.434, 0.875, 1.79, and 3.39 mg/L; Final measured were 85-91% of nominal concentrations
Unit	mg/L
NOEC, LOEC or NOEL, LOEL	72 hr EC50=2.81 mg/L based on average specific growth rate; 72-hr EC50=1.35 mg/L calculated using the number of cells/mL; 72-hr EC50= 1.01 mg/L using the area under the growth curve. The 72-hr NOEC=0.118 mg/L based on number of cells/mL
Biological observations	Control algal populations grew at an acceptable rate (220,000 cells/ml) after 72 hours. Incubation temperatures were in the range from 23.4 to 23.6 C over the 72 hours and pH was unchanged by the test substance. At the conclusion of the test, samples of test media from each test vessel with maximal growth inhibition were combined with fresh media. After 48 hours incubation the number of cells increased from 410 cells/mL to 230,000 cells/mL at 3.39 mg/L suggesting that the toxic effects were algistatic.
Appropriate statistical evaluations?	EC50 values determined by weighted least squares non-linear regression (Bruce and Versteeg, 1992); NOEC was determined using a one-way analysis of variance (ANOVA) and Bonferroni's test (Gulley et al. 1990)
Conclusion remarks	The acute toxicity of estragole measured as a 50% decrease in growth and reproduction of freshwater algae was estimated to be 72 hr EC50=2.81 mg/L based on average specific growth rate; 72-hr EC50=1.35 mg/L calculated using the number of cells/mL; 72-hr EC50= 1.01 mg/L using the area under the growth curve. The 72-hr NOEC=0.118 mg/L based on number of cells/mL
Reliabilities	Reliability code 1. Reliable without restrictions.
Remarks for Data Reliability	OECD 201 Guideline study
References	Boeri R.L.. (2003) The growth and reproduction toxicity test with estragole and freshwater alga, <i>Selenastrum capricornutum</i> . OECD 201. Study No. 2503-FF. Private Communication to FFHPVC. Unpublished Report.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Green algae

Exposure Period	96 hour
Remarks for Test Conditions	Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 °C.
Endpoint Value	EC50 = 3.681mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U S Environmental Protection Agency.

4 HUMAN HEALTH TOXICITY

4.1 Acute Toxicity

Substance Name	Estragole (>95%)
CAS No.	140-67-0
Method/guideline	Litchfield and Wilcoxon, 1949
Test Type	Oral LD50
GLP	No
Year	1964
Species/strain	Rat/Osborne Mendel
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	None
Route of Administration	Oral-Gavage
Remarks for Test Conditions	The test material was administered to 5 male and 5 female Osborne-Mendel rats per dose. Animals were fasted for 18 hours prior to dosing. All doses were given by intubation. Observations for two weeks included mortality and/or systemic effects. LD50 results were calculated using Litchfield-Wilcoxon (1949).
Value LD50 or LC50 with confidence limits	1820 mg/kg bw 95% confidence limits = 1670-1980 mg/kg bw.
Number of deaths at each dose level	Not given
Remarks for Results	Death from 4 hours to 8 days. Toxic signs included depression, coma, rough fur, wet posterior and porphyrin-like deposits around eye reported as toxic sign. No necropsy performed.
Conclusion remarks	The oral LD50 was calculated to be 1820 mg/kg bw with 95% confidence limits = 1670-1980 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L. and Fitzhugh O.G. (1964) Food flavorings and compounds of related structure I. Acute oral toxicity. Food and Cosmetics Toxicology, 2(3), 327-343.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Not given
Test Type	Oral LD50
GLP	No
Year	1972
Species/strain	Rabbit/New Zealand White
Sex	Not reported
Number of animals per sex per dose	10
Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	Ten New Zealand white rabbits were administered the test substance on their clipped abraded abdominal skin. Observations made for mortality and toxic effects.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	0/10 deaths
Conclusion Remarks	The dermal LD50 was reported to be greater than 5000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972a) Acute dermal toxicity of estragole in rabbits. Unpublished report to RIFM.

Substance Name	Estragole (assay:>95%)
CAS No.	140-67-0
Method/Guideline	Litchfield and Wilcoxon, 1949
Test Type	Oral LD50
GLP	No
Year	1964
Species/strain	Mouse

Sex	Not reported
Vehicle	None
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Oral doses of test substance given to mice on full stomachs. Doses administered <i>via</i> intubation. Mice observed for two weeks.
Value LD50 or LC50 with confidence limits	1250 mg/kg bw 95% confidence limits = 812-1920 mg/kg bw
Number of deaths at each dose level	Not given
Remarks for Results	Death from 1 hour to 4 days. Toxic signs included depression and coma at higher doses.
Conclusion Remarks	The oral LD50 was calculated to be 1250 mg/kg bw with 95% confidence limits = 812-1920 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L. and Fitzhugh O.G. (1964) Food flavorings and compounds of related structure I. Acute oral toxicity. Food and Cosmetics Toxicology, 2(3), 327-343.

Substance Name	Estragole
CAS No.	140-67-0
Method/Guideline	Not given
Test Type	Oral LD50
GLP	No
Year	1972
Species/strain	Rat/Wistar
Sex	Male
Number of animals per sex per dose	10
Vehicle	None
Route of Administration	Oral
Remarks for Test Conditions	Ten male albino Wistar rats per group were used. Animals were fasted for a minimum of 16 hours prior to administration of the test material. Animals weighed 200-250 grams. Following dosing the animals received food and water <i>ad libitum</i> . Observations for mortality were made at 1 and 6 hours after dosing and daily thereafter for 14 days. Toxic effects were also

	observed. Gross necropsies were performed on all survivors.
Value LD50 or LC50 with confidence limits	1230 mg/kg bw 95% Confidence Limits (1080-1380 mg/kg bw)
Number of deaths at each dose level	820 mg/kg bw: No observable effects, 1030 mg/kg bw: 2/10 deaths, 1230 mg/kg bw: LD50, 1280 mg/kg bw: 6/10 deaths; 1600 mg/kg bw 9/10 deaths.
Conclusion Remarks	The oral LD50 was calculated to be 1230 mg/kg bw with confidence limits of 1080-1380 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972b) Acute oral toxicity of estragole in rats. Unpublished report to RIFM.

4.2 Genetic Toxicity

4.2.1 *In vitro* Genotoxicity

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99.%
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1982
Species/Strain	<i>Salmonella typhimurium</i> TA 98, TA 100, TA 1535, and TA 1537
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	30-300 micrograms/plate
Statistical Methods	Student's t test
Remarks for Test Conditions	The assays with S9 were conducted using the pre-incubation method, while the assays without S-9 were conducted using the plate incorporation method. The positive controls were 9-aminoacridiine (9-AAc) with TA1535 and TA1537 (5 ug/plate) and TA1538 (ug/plate); and 5 ug/plate of benzo[a]pyrene (BP)

	with TA98 and TA100. Tests were performed in duplicate
Results	Negative
Cytotoxic concentration	300 ug/plate
Genotoxic Effects	None
Appropriate statistical evaluations?	Yes
Remarks for Results	Estragole was inactive in <i>Salmonella</i> strains TA 1535, TA 1537, TA 98 & TA 100 both in the presence and absence of metabolic activation.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99.9%
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1982
Species/Strain	<i>Escherichia coli</i> WP2 uvrA trp-
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	30-300 micrograms/plate
Statistical Methods	Student's t test
Remarks for Test Conditions	Conducted as in Ames except that histidine was replaced with tryptophan. A mutagenicity test was conducted on <i>Escherichia coli</i> WP2 uvr A trp-, using the plate incorporation method in the absence of S9 metabolic activation. A mixture of the test material in dimethyl sulfoxide (DMSO), 100 ul of an overnight culture of the <i>E. coli</i> strains, and 500 ul of sodium phosphate buffer (0.1 M) was added to test tubes that contained 2 ml of top agar supplemented with 0.1 umole of tryptophan. The tube contents were mixed and then poured onto minimal agar plates.

	The plates were incubated for 37°C for 48 – 72 hours, and the tryptophan-independent colonies were scored following the incubation period. The positive control was 0.01 µg/plate of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2). Tests were performed in duplicate.
Results	Negative
Cytotoxic concentration	300 µg/plate
Genotoxic Effects	None
Appropriate statistical evaluations?	Yes
Remarks for Results	Estragole was inactive in <i>E. coli</i> WPR uvrA both in the presence and absence of metabolic activation.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	No
Year	1977
Species/Strain	<i>Salmonella typhimurium</i> TA 98, TA 100, TA 1535, TA 1537, and TA1538
Metabolic Activation	None
Doses/Concentration	0.2 micromolar or 30 micrograms (calculated based on MW of 148.21)
Statistical Methods	Not given
Remarks for Test Conditions	The solvent used was ethanol. This data was taken from an abstract of a French article.
Results	Negative
Cytotoxic concentration	Not given

Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for Results	Negative
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Dorange J. L., Delaforge M. Janiaud P. and Padieu P. (1977) Mutagenicity of the metabolites of the epoxide diol pathway of safrole and analogs. Study on <i>Salmonella typhimurium</i> . Societe de Biologie de Dijon, 171(5), 1041-1048.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99%
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1991
Species/Strain	<i>Salmonella typhimurium</i> TA 98, TA 100, TA 1535 and TA 1537
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	0.06-0.5 microliters/plate (0.06-0.48 micrograms/plate)
Statistical Methods	Not given
Remarks for Test Conditions	The solvent used was DMSO. The pre-incubation method was used. A modified Ames assay was conducted by the preincubation method, using <i>Salmonella typhimurium</i> strains TA1535, TA1537, TA98 and TA100, in the presence and absence S9 metabolic activation obtained from Aroclor-treated male rats. The test material dissolved in 50 ul dimethyl sulfoxide (DMSO), S9 mix (if desired), and the bacterial strains were preincubated together for 20 minutes at 37°C. Next, 2 ml of soft agar was added and the mixture was poured over 30 ml of minimal glucose agar in a Petri dish. Following incubation at 37°C for 2 hours, the revertants per plate were counted. Assays were run in duplicate. Positive controls not given.
Results	Negative

Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion Remarks	No evidence of mutagenic activity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Zani F., Massimo G., Benvenuti S., Bianchi A., Albasini A., Melegari M., Vampa G., Bellotti A., Mazza P. (1991) Studies on the genotoxic properties of essential oils with <i>Bacillus subtilis</i> rec-assay and <i>Salmonella</i> microsome reversion assay. <i>Planta Medica</i> , 57(3), 237-241.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Ames
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1987
Species/Strain	<i>Salmonella typhimurium</i> TA 97, TA 98, TA 100, TA 1535, and TA 1537
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	1-200 micrograms/ml
Statistical Methods	Not given
Remarks for Test Conditions	The pre-incubation method was used. The vehicle was DMSO.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for results	Estragole was inactive in <i>Salmonella</i> strains TA 1535, TA 1537, TA 97, TA 98 & TA 100 both in the presence and absence of metabolic activation system. A preincubation modification of the <i>Salmonella</i> /microsome test was conducted in the presence and

absence of liver S9 from Aroclor-induced male Sprague-Dawley rats or male Syrian hamsters. Strains TA98, TA100, TA1535 and TA1537 and/or TA97 were used. Concurrent solvent and positive controls were run with each trial. The positive controls in the absence of metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation was 2-aminoanthracene for all strains. Trials were run in duplicate.

Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Zeiger E, Anderson B., Haworth S. Lawlor T., Mortelmans K. and Speck W. (1987) Salmonella mutagenicity tests: III. Results from testing 255 chemicals. Environmental Mutagenesis 9(9), 1-109.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1982
Species/Strain	<i>Salmonella typhimurium</i> TA 98, TA 100, TA 1535, TA 1537, and TA1538
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	0.05 -50 micrograms/plate
Statistical Methods	Not given
Remarks for Test Conditions	<p>An Ames plate incorporation test was conducted with and without metabolic activation in strains TA1535, TA100, TA1537, TA1538 and TA98. The vehicle and negative control was ethanol. Metabolic activation was provided by liver S9 prepared from Aroclor 1254-induced rats. The positive control was 10.0 ug/plate 2-aminoanthracene.</p> <p>For strain TA1538, metabolic activation was provided by 3'-phosphoadenosine-5'-phosphosulfate (PAPS) and with and without liver S9 prepared from Aroclor 1254-induced rats.</p>
Results	No mutagenic effects except a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and PAPS (3'-phosphoadenosine 5'-

	phosphosulfate) cofactor.
Cytotoxic concentration	Not given
Genotoxic Effects	See remarks for results.
Appropriate statistical evaluations?	None given
Remarks for results	No mutagenic effects except a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and PAPS (3'-phosphoadenosine 5'-phosphosulfate) cofactor. The authors proposed that mutagenic response was related to the formation of the sulfate ester of an active metabolite. All other strains of <i>Salmonella typhimurium</i> were not mutagenic in assays using PAPS.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	To L.P., Hunt T.P. and Andersen M.E. (1982) Mutagenicity of trans-anethole, estragole, eugenol and safrole in the Ames <i>Salmonella typhimurium</i> assay. Bulletin of Environmental Contamination and Toxicology, 28(6), 647-654.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	No
Year	1979
Species/Strain	<i>Salmonella typhimurium</i> TA 98, and TA 100
Metabolic Activation	Metabolic activation was provided by hepatic S13 fractions prepared from Aroclor 1254-treated CD rats
Doses/Concentration	The doses were 5-20 umoles/plate in TA100 and up to 30 umoles/plate in TA98
Statistical Methods	Not given
Remarks for Test Conditions	The vehicle and negative control was ethanol. Positive controls were not included. An Ames test was conducted with and without metabolic activation in strains TA100 and TA98 (provided by Ames). The vehicle and negative control was ethanol. Metabolic activation was provided by hepatic S13 fractions prepared from Aroclor 1254-treated CD rats (Charles River) and an NADPH-generating system
Results	Equivocal. Very weak activity without metabolic activation in

	TA100. Activity increased in TA100 with activation. No effect was seen in TA98.
Cytotoxic concentration	Not given
Genotoxic Effects	Positive in TA100. Negative in TA98.
Appropriate statistical evaluations?	None given
Remarks for results	Very weak activity without metabolic activation in TA100. Activity increased in TA100 with activation. No effect was seen in TA98. The doses were 5-20 umoles/plate in TA100 and up to 30 umoles/plate in TA98. The metabolites of estragole (1'-hydroxyestragole and estragole-2',3'-oxide) were positive in strains TA100 and TA1535, but were negative in strain TA98
Conclusion Remarks	Equivocal.
Data Qualities Reliabilities	Reliability code 3. Not reliable.
Remarks for Data Reliability	Code 3. Does not meet important criteria of current standard methods.
References	Swanson A.B., Chambliss D.D., Blomquist J.C., Miller E.C. and Miller J.A. (1979) The mutagenicities of safrole, estragole, eugenol, <i>trans</i> -anethole, and some of their known or possible metabolites for <i>Salmonella typhimurium</i> mutants. Mutation Research, 60(2), 142-153.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99.9%
Method/guideline	Rec assay performed according to Kada <i>et al.</i> , 1980
Test Type	DNA repair
System of Testing	Bacterial
GLP	Ambiguous
Year	1982
Species/Strain	<i>Bacillus subtilis</i> H17 Rec + and M45 Rec -
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/Concentration	4 mg/disk
Statistical Methods	Student's t test
Remarks for Test Conditions	Zones of killing with both strains (Rec + and Rec -) were measured and the difference between them was taken as the rec effect. Conducted according to Kada <i>et al.</i> except that 2 E5 spores used instead of 2 E6 to increase the sensitivity of the

	test.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion Remarks	The test substance did not induce DNA repair.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	UDS
Test Type	DNA repair
System of Testing	Mammalian
GLP	Ambiguous
Year	1990
Species/Strain	Hepatocytes from Male Fisher 344 rats
Metabolic Activation	No
Doses/Concentration	0.148-1480 mg (10 ⁻⁶ to 10 ⁻² M)
Statistical Methods	Not given
Remarks for Test Conditions	<p>Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance.</p> <p>Alkenylbenzene flavors were tested in the unscheduled DNA synthesis (UDS) assay in freshly isolated hepatocytes from male Fischer 344 rats in primary culture. UDS was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with flavor chemicals in Dimethyl sulfoxide (DMSO). Cell viability was assessed by determining the amount of cytoplasmic lactate dehydrogenase (LDH) leakage into overnight cell cultures. In this case, hepatocyte cultures were treated as for the UDS assay, but omitting [3H]thymidine. Positive control was 2-</p>

acetylaminofluorene.

Results	Positive. Dose related increase in UDS. 2.7 times greater than control.
Cytotoxic concentration	5 X 10 ⁻³ M
Genotoxic Effects	Positive
Remarks for results	No UDS observed at concentrations at or above 5 X 10 ⁻³ M at which there was significant LDH leakage indicating cytotoxicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Howes A.J., Chan V.S.W. and Caldwell J. (1990) Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. Food and Chemical Toxicology, 28(8), 537-542.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 99%
Method/guideline	UDS
Test Type	DNA repair
System of Testing	Mammalian
GLP	Ambiguous
Year	1992
Species/Strain	Hepatocytes from Male Fisher 344 rats
Metabolic Activation	No
Doses/Concentration	10 ⁻⁴ to 10 ⁻³ M (14.8-148 mg)
Statistical Methods	Not given
Remarks for Test Conditions	Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance. A ratio of 1.5 is considered to be a positive response. The ability of the test material to induce unscheduled DNA synthesis in hepatocytes derived from male Fischer 344 rats (155-240 g) was evaluated. Vehicle was DMSO. Positive control was 2-Acetamidofluorene 10(5) M. Nuclear DNA [3H]thymidine incorporation was used as a measure of unscheduled DNA synthesis. Cytotoxicity was assessed by lactate dehydrogenase leakage.

Results	Positive. Dose related increase in UDS. 2.68 +/- 0.93 times greater than control at 5 X 10 ⁻³ M
Cytotoxic concentration	5 X 10 ⁻³ M
Genotoxic Effects	Positive
Appropriate statistical evaluations?	Not given
Remarks for results	No UDS observed at concentrations above 5 X 10 ⁻³ M at which there was significant LDH leakage indicating cytotoxicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Chan V.S.W. and J. Caldwell. (1992) Comparative induction of unscheduled DNA synthesis in cultured rat hepatocytes by allylbenzenes and their 1'-hydroxy metabolites. Food and Chemical Toxicology, 30, 831-836.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 99%
Method/guideline	UDS
Test Type	DNA repair
System of Testing	Mammalian
GLP	Ambiguous
Year	1992
Species/Strain	Hepatocytes from Wistar rats
Metabolic Activation	No
Doses/Concentration	0.01-10 mM (1.48-1482 mg)
Statistical Methods	Not given
Remarks for Test Conditions	Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance. Fifty hepatocytes per slide from 3 different parallel cultures were evaluated for UDS. Results reconfirmed with independent repeat experiment. Net grain values determined by subtracting the mean of three cytoplasm grain counts from the nuclear grain counts. Cytotoxic effects qualified by determination of necrotic cells. UDS positive cells determined to be percentage of cells with five or more net grains increase over negative controls. The unscheduled DNA synthesis (UDS) assay was conducted in cultures of primary rat hepatocytes using test material dissolved in dimethyl sulfoxide. Hepatocytes were

isolated from 8-10 week old Wistar rats. Experiment was terminated after 18 hours of culture. Grains were counted with a microscope combined with an Artek counter (Model 982b). Cytotoxic effects were qualified by determination of necrotic cells.

Results	Positive at all concentrations. 0.01 millimolar, 0.1 millimolar, 1 millimolar Lethality: 10 millimolar
Cytotoxic concentration	1 X 10 ⁻² M
Genotoxic Effects	Positive
Appropriate statistical evaluations?	None given
Remarks for results	Positive.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential in vitro and in vivo of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 99%
Method/guideline	Chromosomal aberrations in V79 cells
Test Type	Chromosomal Aberration
System of Testing	Mammalian
GLP	Ambiguous
Year	1992
Species/Strain	V79 cells from Wistar rats
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	10 ⁻⁵ to 10 ⁻³ M (1.48 mg- 148 mg)
Statistical Methods	Not given
Remarks for Test Conditions	Chromosomal aberrations determined in V79 cells with and without metabolic activation. Cultures harvested 18 hours after treatment. (2 hour treatment with S9 mix). Test material in dimethyl sulfoxide was tested for the induction of chromosome

	aberrations in V79 cells with and without metabolic activation (rat liver S9) or in co-culture with primary rat hepatocytes. Experiments were terminated after 18 hours of culture. Mitomycin C (MMC) and cyclophosphamide (CP) were used as positive controls. The experiments were run with two cultures in parallel.
Results	Negative-dose was 10(-5) to 10(-3) molar with and without metabolic activation (rat liver S9) or in co-culture with primary rat hepatocytes.
Genotoxic Effects	Negative
Appropriate statistical evaluations?	Chi square distribution
Remarks for results	Negative
Conclusion Remarks	Estragole did not induce chromosomal aberrations in V79 cells with and without metabolic activation.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential <i>in vitro</i> and <i>in vivo</i> of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99%
Method/guideline	Rec assay performed according to Mazza <i>et al.</i> , 1982
Test Type	DNA repair
System of Testing	Bacterial
GLP	Ambiguous
Year	1991
Species/Strain	<i>Bacillus subtilis</i> PB1652 and PB1791
Metabolic Activation	None
Doses/Concentration	10-30 microliters (9.6-29 micrograms/plate)
Statistical Methods	Not given
Remarks for Test Conditions	A positive DNA damaging activity was assumed when the ratio of the inhibition zone of the rec- mutant and that of the parental rec + strain exceeded the value of 1.2. The test material was applied to a sterile filter paper disk (9 mm diameter) which was placed on the surface of nutrient agar plates seeded with the

tester strains. After an overnight incubation at 37°C, the diameter of the inhibition zones which formed around the disk, were measured with a Vernier caliper. Methyl methanesulfonate (MMS), mitomycin C (MIT C), adriamycin (ADR) were used as positive controls, while ampicillin (AMP) and chloramphenicol (CAF) as negative controls.

Results	Positive
Cytotoxic concentration	Not given
Genotoxic Effects	Positive
Appropriate statistical evaluations?	None given
Remarks for Results	Positive
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Zani F., Massimo G., Benvenuti S., Bianchi A., Albasini A., Melegari M., Vampa G., Bellotti A., Mazza P. (1991) Studies on the genotoxic properties of essential oils with <i>Bacillus subtilis</i> rec-assay and <i>Salmonella</i> microsome reversion assay. <i>Planta Medica</i> 57(3), 237-241.

4.2.2 *In vivo* Genotoxicity

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation
GLP	No
Year	1984
Species/Strain	Mouse/CD-1
Sex	Female
Route of Administration	Intraperitoneal
Doses/Concentration	2 or 10 mg/mouse
Exposure Period	Single dose
Remarks for Test Conditions	Groups of 3-4 female CD-1 mice were given an intraperitoneal injection of 0, 2 or 10 mg estragole/mouse in 0.1 ml triolein. Twenty-four hours following treatment, mice were killed and livers were collected and frozen at -80 deg C. DNA was isolated from the frozen livers using a rapid solvent-extraction procedure and quantitated spectrophotometrically. DNA was digested and 32P-labelled. Labelled adducts were purified by reversed phase thin layer chromatography and contact transfer to polyethyleneimine-cellulose. Adduct levels (as reactive adduct labelling [RAL]) were determined (adduct spot/normal nucleotidesx600) and covalent binding indices (CBI) were calculated (umol of anethole bound/mol of DNA nucleotides divided by mmol of anethole administered/kg bw).
Genotoxic effects	Positive
NOEL (C)/ LOEL (C)	LOEL: 2 mg/kg bw
Remarks for Results	DNA adducts were detected at both dose levels.
Conclusion Remarks	Estragole showed binding potential to mouse-liver DNA.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Randerath, K., Haglund, R.E., Phillips, D.H., and Reddy, M.V. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis 5(12): 1613-1622.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation
GLP	No
Year	1981
Species/Strain	Mouse/B6C3F1
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	14 mg/kg bw
Exposure Period	Single dose
Remarks for Test Conditions	In a study designed to detect DNA adduct formation of estragole, 9-day old male or female B6C3F1 mice (mean weight, 6g) were given intraperitoneal injections of 0.5 mmol (14 mg/kg) of labeled estragole and sacrificed after 23 hours.
NOEL (C)/ LOEL (C)	LOEL: 14 mg/kg bw
Genotoxic effects	Positive
Remarks for Results	DNA adducts were detected.
Conclusion Remarks	Estragole showed binding potential to mouse-liver DNA.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Phillips, D.H., J.A. Miller, E.C. Miller, and B. Adams. (1981) Structures of the DNA adducts formed in mouse liver after administration of the proximate hepatocarcinogen 1'-hydroxyestragole. Cancer Research, 41, 176-186.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 98%
Method/guideline	<i>in vivo</i> UDS
Test Type	DNA repair
GLP	Ambiguous

Year	1994
Species/Strain	Rat/Wistar
Sex	Male
Route of Administration	Gavage
Doses/Concentration	500, 1,000 or 2,000 mg/kg bw
Exposure Period	Single dose
Remarks for Test Conditions	Test material in peanut oil was administered to male Wistar rats at dose levels of 500, 1,000 or 2,000 mg/kg bw. Hepatocytes isolated from sacrificed rats 4 or 12 hours after the single dose. After 18 hours of culture, fifty hepatocytes per slide were evaluated for UDS. Net grain values obtained by subtracting the mean of three cytoplasm grain counts from the nuclear grain counts. Cytotoxic effects determined by the number of necrotic cells. Cells considered positive for UDS if percentage of cells with five or more net grains increased over the negative concurrent control values.
Genotoxic effects	500 mg/kg bw- weak effect; 1,000 mg/kg weak effect; 2,000 mg/kg clear positive effect at this dose level. No difference between cells isolated at 4 hours and those isolated at 12 hours.
NOEL (C)/ LOEL (C)	LOEL: 500 mg/kg bw
Appropriate statistical evaluations?	None given
Remarks for Results	Only a very slight increase in net grain values reported for the 500 and 1000 mg/kg bw dose levels. The highest dose levels produced clear increases.
Conclusion Remarks	The authors characterize the results seen at the two lowest dose levels as being very slight increases and given the lack of appropriate statistical analyses, these results are considered questionable.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential in vitro and in vivo of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation

GLP	No
Year	1984
Species/Strain	Mouse/B6C3F1
Sex	Male
Route of Administration	Intraperitoneal
Doses/Concentration	0.25, 0.5, 1.0, and 3.0 mmol
Exposure Period	23, 29 or 43 days
Remarks for Test Conditions	32P-post-labelling analysis was used to detect test material-DNA adducts in livers of treated mice. B6C3F1 male mice received 0.25, 0.5, 1.0 and 3.0 umol of test material on days 1, 8, 15 and 22, respectively, after birth. Groups of 3 mice were killed for analysis on days 23, 29 and 43 (i.e. 1, 7, and 21 days after the final injection) and the livers removed and weighed. Vehicle was trioctanoin.
Genotoxic effects	Positive
Remarks for Results	DNA adducts were detected.
Conclusion Remarks	Estragole showed binding potential to mouse-liver DNA.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Phillips D.H., Reddy M.V. and Randerath K. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. II. Newborn male B6C3F1 mice. Carcinogenesis, 5(12), 1623-1628.

4.3 Repeated Dose Toxicity

Substance Name	Methyl eugenol (surrogate for estragole)
CAS No.	93-15-2
Remarks for Substance	Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 98%
Method/guideline	OECD Guideline 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents)
GLP	Yes
Year	2004

Species/strain	Rat/F344/N
Sex	Male and Female
Route of Administration	Oral-Microencapsulated in the diet
Doses/concentration Levels	0, 1, 5, or 50 mg/kg bw/d;
Exposure Period	28 days
Frequency of Treatment	Continuous
Control Group	Yes
Post Exposure	None
Remarks for Test Conditions	<p>The study was designed to investigate the systemic toxicity of the test material. It is based on the recommendations of the OECD Guidelines for Testing of Chemicals No. 407 "Repeated Dose 28 Day Oral Toxicity Study in Rodents" (Adopted 27 July 1995)</p> <p><i>Dietary</i></p> <p>The test material was administered continuously throughout the treatment period by dietary admixture to three groups each of ten male and ten female Fischer 344 F344/NHsd strain rats, for twenty-eight consecutive days, at dose levels of 1, 5 and 50 mg/kg/day. Two control groups each of five males and five females was treated with untreated diet only or untreated diet plus microencapsulated matrix (50 mg/kg bw/day).</p> <p><i>Gavage.</i></p> <p>The test material was administered by gavage to ten male and ten female Fischer 344 F344/NHsd strain rats, for twenty-eight consecutive days, at a dose level of 50 mg/kg/day. A control group of five males and five females was dosed with vehicle alone (distilled water). Clinical signs, bodyweight development, urine samples, food and water consumption were monitored during the study. Hematology, urinalysis and blood chemistry were evaluated for all animals at the end of the study. Blood samples of test and control group animals were taken once prior to the start of treatment and again on Day 28. Plasma was separated and stored at approximately -20°C prior to dispatch to Dr Paul Carmichael, Imperial Collage, London. Biochemical studies on DNA and protein adduct formation and PCNA cell proliferation studies were performed on liver and forestomach tissues</p> <p>All animals were subjected to a gross necropsy examination and at necropsy blood samples were collected, allowed to clot and serum was then separated and stored at approximately -20°C prior to despatch to Dr Paul Carmichael, Imperial College, London.</p> <p>Preliminary histopathological evaluation of the stomach and</p>

liver from all animals was performed. Additional samples of the liver and stomach from all animals were stored in liquid nitrogen prior to despatched to Paul Ellis and Dr Paul Carmichael, Imperial College, London.

NOAEL(NOEL)	50 mg/kg bw/d in the diet
LOAEL(LOEL)	Not determined
Toxic Response/effects by Dose Level	See remarks for results.
Appropriate statistical evaluations?	Yes
Remarks for results	<p>There were no unscheduled deaths during the study. One Dietary female treated with 50 mg/kg/day developed a damaged tail from Day 14 onwards. Four high dose gavage males showed fur loss from Day 4 onwards and a further two high dose gavage males showed fur loss from Day 11 onwards. High dose gavage males showed a slight reduction in bodyweight gain during Week 1 of treatment. There was no effect on bodyweight in any group of males and female by either the dietary or gavage route of administration. There were no differences between food intake or food efficiency uptake for any of the treated groups compared to controls. Haematological examination, blood chemical determinations and urine analysis revealed a slight increase in cholesterol in gavage males at the 50 mg/kg bw/d per day dose level.</p> <p>Dietary animals treated with 1, 5 or 50 mg/kg/day showed a slight reduction in liver weight relative to bodyweight and absolute weight (males only). There was no treatment-related organ weight changes detected in gavage animals treated with 50 mg/kg/day. One dietary female treated with 1 mg/kg/day showed small nodules on the median lobe attached to the diaphragm of the liver. Three high dose gavage males showed fur loss and one high dose dietary female had a damaged tail. There were no further macroscopic abnormalities detected. There were no treatment-related changes detected. P³²-postlabeling experiments indicate that at detection limits of 1/10⁹ adducts, no methyl eugenol-DNA adduct are detected at 1 mg/kg bw/day and there is equivocal evidence of adduct formation at 5 mg/kg bw/d</p>
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.

References

Jones L. (2003) Twenty-eight day repeated dose oral (dietary and gavage) toxicity study in the rat. SPL Project No. 1834/002. Unpublished report to FEMA.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 98%
Method/guideline	National Toxicology Program. 90-Day oral toxicity study
GLP	Yes
Year	2005
Species/strain	Mouse/B6C3F1
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 37.5, 75, 150, or 300 mg/kg bw/d for females and 0, 37.5, 75, 150, 300, or 600 mg/kg bw/d for males
Exposure Period	93 days
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Post Exposure	None
Remarks for Test Conditions	Groups of 10 female mice each were administered 0, 37.5, 75, 150, or 300 mg/kg bw/d estragole via gavage once per day, five days per week for 93 days. Males were administered an additional dose level of 600 mg/kg bw/d. Animals were housed five per cage and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination (day 93). At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epididymus, testes, pancreas, haemopoietic system, olfactory epithelium, and kidney.
NOAEL(NOEL)	75 mg/kg bw/d
LOAEL(LOEL)	150 mg/kg bw/d
Toxic Response/effects by Dose Level	See remarks for results.

Appropriate statistical evaluations?	Yes
Remarks for results	<p>In male mice, survival was 100% for all dosed groups except for the 600 mg/kg bw/d level. Statistically significant decreases in body weight were recorded for the 300 and 600 mg/kg bw/d dosed groups compared to that of controls. Haematology examinations revealed decreases in erythrocytes and increases in the number of leucocytes, lymphocytes, reticulocytes, and platelets but only at the 300 and 600 mg/kg bw/d dose levels. Organ weight changes included increased relative (to body weight) liver and decreased body weight at 300 and 600 mg/kg bw/d.</p> <p>Histopathological examination revealed liver alterations at 300 and 600 mg/kg bw/d including oval cell hyperplasia, hepatocyte hypertrophy, hepatocyte degeneration all of which were described as being minimal or mild in severity.</p> <p>Effects in female mice were less pronounced than in males. Survival was 100% for all dosed groups. Statistically significant decreases in body weight were recorded for the 150 and 300 mg/kg bw/d dosed groups compared to that of controls. Haematology examinations revealed decreases in erythrocytes and increases in the number of platelets, leucocytes, lymphocytes, and reticulocytes 150 and 300 mg/kg bw/d. Organ weight changes increased absolute and relative (to body weight) liver weight at 300 mg/kg bw/d.</p> <p>Histopathological examination revealed no alterations to the liver or any other organ or tissues at levels up to and including 300 mg/kg bw/d.</p>
Conclusion Remarks	Based primarily on the histopathologic changes, a NOAEL of 75 mg/kg bw/d and a LOAEL of 150 mg/kg bw/d was reported for the subchronic toxicity of estragole in male and female mice.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (2005) Toxicology and of estragole in F344/N Rats and B6C3F1 mice. U.S. Dept of Health and Human Services. NIH Publication No., not assigned.
Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 98%
Method/guideline	National Toxicology Program. 90-Day oral toxicity study
GLP	Yes
Year	2005

Species/strain	Rat/F344/N
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 37.5, 75, 150, 300, or 600 mg/kg bw/d
Exposure Period	93 days
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Post Exposure	None
Remarks for Test Conditions	Groups of 10 male and 10 female rats each were administered 0, 37.5, 75, 150, 300, or 600 mg/kg bw/d estragole via gavage once per day, five days per week for 93 days. Animals were housed five per cage and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination (day 93). At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epididymus, testes, pancreas, hemapoietic system, olfactory epithelium, and kidney.
NOAEL(NOEL)	Undetermined
LOAEL(LOEL)	37.5 mg/kg bw/d
Toxic Response/effects by Dose Level	See remarks for results.
Appropriate statistical evaluations?	Yes
Remarks for results	In male rats, survival was 100% for all dosed groups. Statistically significant decreases in body weight were recorded for the 300 and 300 mg/kg bw/d dosed groups compared to that of controls. Clinical chemistry changes were limited mainly to the 300 and 600 mg/kg bw/d groups. At 300 and 600 mg/kg bw/d increased levels of blood urea nitrogen, total protein, alanine aminotransferase, bile acid salts, and total iron binding capacity were reported. Additionally at 600 mg/kg bw/d, increases in alkaline phosphatase, bile acids/salts, and succinate dehydrogenase were reported. Haematology examinations revealed decreases in erythrocytes, hemaglobin, hematocrit, mean cell volume and platelet count and increased in the number of leucocytes, lymphocytes, and neutrophils but only at the 300 and 600 mg/kg bw/d dose levels. Organ weight changes included increased absolute and relative (to body weight) liver and kidney weight and decreased body weight and testes weight at 300 and 600 mg/kg bw/d. Absolute and relative heart weight was also increased at 600 mg/kg bw/d

Histopathological examination revealed liver alterations at 37.5 mg/kg bw/d including bile duct hyperplasia, oval cell hyperplasia, hepatocyte hypertrophy, periportal inflammation all of which were described as being minimal effects. Similar alterations at the 75 mg/kg bw per day dose level, were also described as minimal. The severity of hepatic effects at 150 mg/kg bw/d was reported to be mild while the effects at higher dose levels increased in severity (moderate and marked). At 150 mg/kg bw per day and higher dose levels, males also showed evidence of chronic hepatic inflammation, hepatocellular necrosis, oval cell hyperplasia, and hepatic periportal fibrosis. At 600 mg/kg bw/d, cholangiofibrosis was reported in one animal.

Effects in female rats were similar to those in males, but the onset and the severity of the effects were less pronounced than in males. Survival was 100% for all dosed groups. Statistically significant decreases in body weight were recorded for the 300 and 600 mg/kg bw/d dosed groups compared to that of controls. The only consistent clinical observation occurred among high dose animals that appeared gaunt during the course of the study. Clinical chemistry changes were limited mainly to the 300 and 600 mg/kg bw/d groups. At 300 and 600 mg/kg bw/d increased levels of creatine kinase, succinate dehydrogenase, alanine aminotransferase, and total iron binding capacity were reported. Decreased serum iron was also reported at the two highest dose levels. Additionally at 600 mg/kg bw/d, increases in creatinine, total protein, and albumin were reported. Haematology examinations revealed decreases in erythrocytes, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, and reticulocytes. Increases in the number of platelets, leucocytes, lymphocytes, monocytes, and neutrophils were reported beginning at the 75 mg/kg bw/d dose level. These changes were more pronounced at higher dose levels. Organ weight changes included decreased body weights at 300 and 600 mg/kg bw/d, increased absolute and relative (to body weight) liver at dose levels of 37.5 mg/kg bw/d and higher, increased absolute and relative lung weight at 300 and 600 mg/kg bw/d, increased thymus weights at dose levels of 75 mg/kg bw/d and greater, increased heart and right kidney weight at 600 mg/kg bw/d.

Histopathological examination revealed liver alterations at mainly beginning at the 75 mg/kg bw per day dose levels. At 37.5 mg/kg bw/d minimal bile duct hyperplasia, oval cell hyperplasia, eosinophilic foci, and sporadic periportal inflammation was reported. At 75 mg/kg bw/d, the same alterations were observed with greater incidence and severity. Also basophilic foci were reported at this dose level. At 150 mg/kg bw/d, additional alterations included histiocytic cell infiltrate, hepatocyte hypertrophy, mixed cell foci, At 300 and 600 mg/kg bw/day the severity of the effects was greater. Cholangiofibrosis was reported in one animal at 600 mg/kg bw/d.

Conclusion Remarks	Based primarily on the histopathologic changes, a LOAEL of 37.5 mg/kg bw/d was reported for the subchronic toxicity of
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estragole in male and female F344/N rats.

Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (2005) Toxicology and of estragole in F344/N Rats and B6C3F1 mice. U.S. Dept of Health and Human Services. NIH Publication No., not assigned.
Substance Name	Methyl eugenol (surrogate for estragole)
CAS No.	93-15-2
Remarks for Substance	Data is for structurally related substance, methyl eugenol:Purity greater than 98%
Method/guideline	National Toxicology Program. 14 week oral toxicity study
GLP	Yes
Year	2000
Species/strain	Mouse/B6C3F1
Sex	Male and Female
Route of Administration	Oral-Gavage in 0.5% methyl cellulose
Doses/concentration Levels	0, 10, 30, 100, 300 or 1000 mg/kg bw/d
Exposure Period	14 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Post Exposure	None
Remarks for Test Conditions	Groups of 10 female mice each were administered 0, 10, 30, 100, 300, or 1000 mg/kg bw/d methyl eugenol via gavage in 0.5% methyl cellulose once per day, five days per week for 93 days. Animals were housed individually and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination. At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epididymus, testes, pancreas, haemopoietic system, olfactory epithelium, and kidney.
NOAEL(NOEL)	10 mg/kg bw/d

LOAEL(LOEL)	30 mg/kg bw/d based on liver weight increases in males
Toxic Response/effects by Dose Level	See remarks for results.
Appropriate statistical evaluations?	Yes
Remarks for results	In mice, low survival rates were reported at the highest dose level of methyl eugenol in males and females. Mean body weight gains of male and female mice given 300 mg/kg were significantly less than those of the vehicle control. There was a statistical increase ($p < 0.05$) in liver weights in male mice dosed with ≥ 30 mg/kg bw/d and in female mice dosed with 300 mg/kg bw/d compared to those of the respective control groups. Increased incidences of cytologic alteration, necrosis, bile duct hyperplasia, and subacute inflammation were observed in the liver of 1,000 mg/kg male mice and 300 mg/kg and greater female mice. A significant increase in testis weight was observed in male mice receiving 100 or 300 mg/kg/d. There were no significant findings at 10 mg/kg bw/d [NTP, 2000].
Conclusion Remarks	Based primarily on the liver weight changes in males, a NOAEL of 10 mg/kg bw/d and a LOAEL of 30 mg/kg bw/d was reported for the subchronic toxicity of methyl eugenol in male and female mice.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP). (2000) Toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-12) in F344/n rats and B6C3F1 mice (gavage studies). DRAFT NTP-TR-491; NIH Publication No. 98-3950.
Substance Name	Methyl eugenol (surrogate for estragole)
CAS No.	93-15-2
Remarks for Substance	Data is for structurally related substance, methyl eugenol:Purity greater than 98%
Method/guideline	National Toxicology Program. 14 week oral toxicity study
GLP	Yes
Year	2000
Species/strain	Rat/F344/N
Sex	Male and Female
Route of Administration	Oral-Gavage in 0.5% methyl cellulose

Doses/concentration Levels	0, 10, 30, 100, 300 or 1000 mg/kg bw/d
Exposure Period	14 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Post Exposure	None
Remarks for Test Conditions	Groups of 10 female and male rats each were administered 0, 10, 30, 100, 300, or 1000 mg/kg bw/d methyl eugenol via gavage in 0.5% methyl cellulose once per day, five days per week for 14 weeks. Animals were housed individually and fed ad libitum. Body weights and clinical observations were made weekly and on day 1 and at termination. At termination, blood was taken for clinical chemistry and haematology determinations and body and organ weights (heart, brain, liver right kidney, right testes, lungs, and thymus) were recorded. Tissues were prepared and histopathological examination was performed on a wide variety of tissues including the oesophagus, rectum, liver, bile ducts, salivary gland, stromal gland, epididymus, testes, pancreas, haemopoietic system, olfactory epithelium, and kidney.
NOAEL(NOEL)	10 mg/kg bw/d
LOAEL(LOEL)	30 mg/kg bw/d based on liver weight increases in males
Toxic Response/effects by Dose Level	See remarks for results.
Appropriate statistical evaluations?	Yes
Remarks for results	The final mean body weight gains of male rats receiving 300 and 1,000 mg/kg bw/d and all the dosed female rats were significantly ($p=0.01$) less than those of the vehicle control. Liver weights in male rats dosed with ≥ 100 mg/kg bw per day and in female rats dosed with ≥ 300 mg/kg bw/d were significantly higher than those in control rats. Relative liver weights of male rats at 30 mg/kg bw per day were increased (14.08 g) compared to the vehicle controls (12.87 g) but not with respect to untreated controls (13.56 g). A significant increase in testis weight was observed in male rats receiving 1,000 mg/kg/d. Haematological examination revealed a decreased mean packed red cell volume in 300 mg/kg/d male rats and in male and female rats receiving 1,000 mg/kg/d. There were also increased platelet counts and increased alanine aminotransferase and sorbitol dehydrogenase activities in male and female rats receiving ≥ 100 mg/kg/d. Additionally, hypoproteinemia, hypoalbuminemia, and increased bile acid concentrations were evident in male and female rats receiving ≥ 300 mg/kg/d. An increase in the incidence of adrenal gland cortical hypertrophy and/or cytoplasmic alteration in the submandibular gland occurred in 100 mg/kg or greater male and female rats. The incidences of atrophy and chronic inflammation (chronic gastritis) of the glandular stomach

	mucosa were significantly increased in male and female rats administered 300 mg/kg or greater, and there was a hepatocellular adenoma in one male rat administered 1,000 mg/kg. There were no significant findings at 10 mg/kg bw/d
Conclusion Remarks	Based primarily on the liver weight changes in males, a NOAEL of 10 mg/kg bw/d and a LOAEL of 30 mg/kg bw/d was reported for the subchronic toxicity of methyl eugenol in male and female rats.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP). (2000) Toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-12) in F344/n rats and B6C3F1 mice (gavage studies). DRAFT NTP-TR-491; NIH Publication No. 98-3950.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 95%
Method/guideline	Carcinogenicity Study
GLP	No
Year	1983
Species/strain	Mice/CD-1
Sex	Male and Female
Route of Administration	Gavage
Doses/concentration Levels	0, 370 mg/kg bw
Exposure Period	Five weeks
Frequency of Treatment	Twice a week for 10 doses
Control Group	Yes
Post Exposure	13 months
Remarks for Test Conditions	Male (55) and female (49) CD-1 mice were administered 370 mg/kg of estragole by gavage twice a week for ten doses beginning at 4 days of age. The mice were weaned at 35 days of age following the last intubation.
NOAEL(NOEL)	Not determined
LOAEL(LOEL)	370 mg/kg bw

Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	Hepatomas were observed as early as 11 months. At 14 months, 73% of the males (3.5 hepatomas/mouse) and 24% of control males (0.6 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in females (9%, 0.1 hepatomas/mouse) was not statistically different from control females (2%, 0.02 hepatomas/mouse) [Miller <i>et al.</i> , 1983]
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research, 43, 1124-1134.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	The metabolites, 1-hydroxyestragole and estragole epoxide, were also evaluated.
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1983
Species/strain	Mice/CD-1
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/concentration Levels	9.45 mmol/mouse of estragole or estragole epoxide or 1.87 mmoles/mouse of 1'-hydroxyestragole by intraperitoneal injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04 mmol/mouse, respectively.
Exposure Period	22 days
Frequency of Treatment	Days 1, 8, 15, and 22 of life
Control Group	Yes
Post Exposure	13 months
Remarks for Test Conditions	Male (50) and female (50) CD-1 mice were administered a total dose of 9.45 mmol/mouse of estragole or estragole epoxide or 1.87 mmoles/mouse of 1'-hydroxyestragole by intraperitoneal

	injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04 mmol/mouse, respectively. The mice were weaned at 22 days of age.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	At 12 months, 65% of the mice receiving estragole exhibited hepatomas (1.7 hepatomas/mouse) versus 26% of controls (0.5 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in mice given estragole epoxide (40%, 0.6 hepatomas/mouse) was not statistically different from control (26%, 0.5 hepatomas/mouse). For 1'-hydroxyestragole, 93% of the mice receiving the test substance (2.7 hepatomas/mouse) and 15% of control males (0.2 hepatomas/mouse) exhibited hepatomas [Miller <i>et al.</i> , 1983]
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research, 43, 1124-1134.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for metabolite, 1-hydroxyestragole
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1987
Species/strain	Mice/Male C57BL/6J x C3H/HeJ F1
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/concentration Levels	Dose levels were 0.1 mmol on Day 1, 0.04 mmol on days 8 and 15, and 0.08 mmol on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively.
Exposure Period	22 days
Frequency of Treatment	Days 1, 8, 15, and 22 of life

Control Group	Yes
Post Exposure	14 months
Remarks for Test Conditions	In a study using a hybrid strain of B6C3F1 mice, and the parent strain, C3H/He male and female mice and C57BL/6 male and female mice, the mice were given intraperitoneal injections of 1'-hydroxyestragole on days 1, 8, 15, and 22. Dose levels were 0.1 mmol on Day 1, 0.04 mmol on days 8 and 15, and 0.08 mmol on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively. The experiment was terminated after 14 months.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	The first tumour-bearing mouse was observed at 10 months. At 12 months, 76% of the treated C3H/He male mice (3.0 hepatomas/mouse) and 26% of control mice (0.3 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in C3H/He female mice (6% 0.06 hepatomas/mouse) was not statistically different from those of control females. For C57BL/6 mice, the incidence of hepatomas in treated males was 14% (0.3 hepatomas/mouse) and was 5% (0.07 hepatomas/mouse) in control males. No hepatomas were observed in treated or control B57BL/6 female mice
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wiseman R.W., Miller E.C., Miller J.A. and Liem A. (1987) Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. Cancer Research, 47(9), 2275-2283.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for metabolite, 1-hydroxyestragole
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1987
Species/strain	Mice/Male B6C3F1
Sex	Male
Route of Administration	Intraperitoneal

Doses/concentration Levels	0.10 mmol/g (15 mg/kg) and 0.01 mmol/g (1.5 mg/kg)
Exposure Period	Single dose
Frequency of Treatment	12 days after birth
Control Group	Yes
Post Exposure	12 months
Remarks for Test Conditions	Groups of male B6C3F1 mice were given single intraperitoneal injections of 0.10 mmol/g (15 mg/kg) of body weight of 1'-hydroxyestragole 12 days after birth. Animals were sacrificed after 12 months and incidence of hepatic tumors were measured. A second group of males was given a lower dose of 0.01 mmol/g of body weight.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	A statistically significant increase in the incidence of hepatomas/mouse were observed for both substances at 0.1mmol/g bw, but no significant increase was observed at the low dose of 0.01 mmol/g bw (1.5 mg/kg).
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wiseman R.W., Miller E.C., Miller J.A. and Liem A. (1987) Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. Cancer Research, 47(9), 2275-2283.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	The metabolite, 1-hydroxyestragole, was also evaluated.
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1983
Species/strain	Mice/CD-1
Sex	Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0, 2300 or 4600 ppm for estragole and 2500 ppm for 1-hydroxyestragole

Exposure Period	12 months
Frequency of Treatment	Daily
Control Group	Yes
Remarks for Test Conditions	In a multipart study evaluating the carcinogenic potential of allylalkoxybenzene derivatives, groups of CD-1 female mice (mean weight 24 g) were maintained on a diet containing 2300 or 4600 ppm estragole or 2500 ppm 1'-hydroxy estragole. The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2300 ppm and 4600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet. To avoid intolerance the dietary concentration was reduced by 75% for the first 10 days and 50% for the next 10 days. The target diet was then maintained for 12 months.
NOAEL(NOEL)	Not determined
LOAEL(LOEL)	2300 ppm
Actual dose received by dose level and sex	The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2300 ppm and 4600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	Survival at 20 months was slightly lower (68-70%) for estragole fed animals compared to control animals (78%). The average life span of mice given 1'-hydroxyestragole was 13.6 months compared to 18 months in controls. Body weights measured at 1, 4, and 8 months were markedly reduced at 4 and 8 months compared to controls. At 10 months, the incidence of hepatomas was 58% for animals at 2300 ppm estragole, 71% for animals at 4600 ppm estragole and 56% for animals at 2500 ppm of 1'-hydroxyestragole and 0 % in controls. Histopathological examinations revealed portal fibrosis, chronic inflammation and bile duct proliferation in addition to the tumours. Varied number of ceroid-laden histocytes and focal area of hyperplasia and megalocytosis were also reported. Four mice fed 4600 ppm estragole had hepatic angiosarcomas
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research 43, 1124-1134.

Substance Name	Methyl eugenol (surrogate for estragole)
CAS No.	93-15-2
Remarks for Substance	Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 99%
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 491
GLP	Yes
Year	1998
Species/strain	Rat/F344/N
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 37, 75, or 150 mg/kg bw/d; stop exposure group 300 mg/kg bw/d
Exposure Period	105 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Post Exposure	52 weeks for the stop exposure group
Remarks for Test Conditions	Groups of fifty male and fifty female rats each were administered 0, 37, 75 or 150 mg/kg bw/d methyl eugenol in 0.5% methyl cellulose via gavage once per day, five days a week for 105 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study; all vehicle control; all low dose female rats and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostate/testes or ovaries/uterus, esophagus, eyes, femur or sternbrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose male rat groups included adrenal glands, kidney, liver, spleen, and testis.
NOAEL(NOEL)	Not determined

LOAEL(LOEL)	37 mg/kg bw/d
Toxic Response/effects by Dose Level	See remarks for results.
Appropriate statistical evaluations?	Yes
Remarks for results	<p>All 150 and 300 mg/kg males died before the end of the study. Mean body weights of all dosed groups were less than those of the vehicle controls throughout the study. The incidences of liver non-neoplastic lesions in dosed groups of male and females were increased at 6 months, 12 months, and 2 years. There were statistically significant increases in oval cell hyperplasia, hepatocyte hypertrophy, and eosinophilic foci, at all dose levels in male and female rats. At the three highest doses (75, 150, and 300 mg/kg bw per day) atypical focal bile duct hyperplasia, focal cystic degeneration, and mixed cell foci were observed, more in males than females. Many of the same non-neoplastic lesions of the liver were reported in the 300 mg/kg bw groups of male and female rats at both 6 and 12 months in the stop-exposure group. Non-neoplastic lesions of the glandular stomach included statistically significant increases in mucosal atrophy at all dose levels and neuroendocrine hyperplasia at the three highest dose levels in females and at all dose levels in males. There was a significant increase in the incidence of nephropathy in females at 300 mg/kg, and the incidence of renal tubule hyperplasia was greater in the greater than 75 mg/kg groups than in the vehicle control.</p> <p>Methyl eugenol-related liver neoplasms occurred in all dosed groups and comprised hepatocellular adenomas and carcinomas, hepatocholangiomas, and hepatocholangiocarcinomas. There was a statistically significant increase (P equals 0.049 in males and P equals 0.017 in females at 37 mg/kg bw; P less than 0.001 for all other treated groups) in the incidence of hepatocellular adenomas and carcinomas in all dose groups of males and female rats. Hepatocholangiomas and hepatocholangiocarcinomas were reported in the 150 mg/kg bw group of males (2/50, 4%) and females (3/49, 6%) and at higher incidence in the 300 mg/kg bw stop-exposure groups of males (13/50, 26%) and females (17/50, 34%). The appearance of cholangiocarcinomas and bile duct dysplasia was said to provide some additional evidence of carcinogenicity based on the rarity of these lesions in F344/N rats (historical incidence, 3/2145, 0.1%).</p> <p>Both benign (3/50, 6%) and malignant (4/50, 8%) neuroendocrine cell neoplasms of the glandular stomach were reported in males at 150 mg/kg bw and in the 300 mg/kg bw stop-exposure group (2/49, 4.1% benign and 2/49, 4.1% malignant). The incidence of these neoplasms was much higher in females at dose levels of 75 mg/kg bw (13/50, 26% benign and 12/50, 24% malignant) and greater.</p>

There were also significant increases in the incidence of: malignant mesothelioma in male rats given greater than 150 mg/kg; and of mammary gland fibroadenoma in 75 and 150 mg/kg males; and fibroma of the subcutaneous tissue in 37 and 75 mg/kg males. These neoplasms were not found in female rats at any dose level.

Conclusion Remarks

The authors determined that under the conditions of these 2-year gavage studies there was clear evidence of carcinogenic activity of methyl eugenol as shown by increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma in male rats. However, because of the evidence of toxicity of methyl eugenol in all groups of rats and mice, the study cannot be recognized as conclusive for carcinogenicity at lower, non-toxic doses. In particular, the hepatic damage undoubtedly altered the metabolism of the compound, and the gastric damage probably altered its absorption.

Data Qualities Reliabilities

Reliability code 1. Reliable without restriction.

Remarks for Data Reliability

Code 1. Guideline study.

References

National Toxicology Program (NTP) (2000) Toxicology and carcinogenesis studies of methyl eugenol in F344/N Rats and B6C3F1 mice. NTP-TR-491. U.S. Dept of Health and Human Services. NIH Publication No. 98-3950.

Substance Name	Methyl eugenol (surrogate for estragole)
CAS No.	93-15-2
Remarks for Substance	Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 99%
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1998
Species/strain	Mice/B6C3F1
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 37, 75, or 150 mg/kg bw/d
Exposure Period	104 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes

Remarks for Test Conditions	<p>Groups of fifty male and fifty female mice each were administered 0, 37, 75 or 150 mg/kg bw/d methyl eugenol in 0.5% methyl cellulose via gavage once per day, five days a week for 104 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study, all vehicle controls, and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostate/testes or ovaries/uterus, esophagus, eyes, femur or sternbrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland.</p>
NOAEL(NOEL)	Not determined
LOAEL(LOEL)	37 mg/kg bw/d (females)
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	<p>Survival of all dosed groups of male mice was similar to that of the vehicle controls. The survival of treated females was significantly less than those reported for control animals. Mean body weights of dosed mice were reported to be "generally less than those of the vehicle controls throughout the studies". In female mice and, to a lesser extent, in male mice there was evidence of hepatotoxicity of methyl eugenol. Significant increases in oval cell hyperplasia, eosinophilic foci, hepatocyte hypertrophy and necrosis, haematopoietic cell proliferation, haemosiderin pigmentation, and bile duct cysts were observed at all dose levels in male and female mice. Non-neoplastic lesions of the glandular stomach included statistically significant increases in hyperplasia, ectasia, atrophy at all dose levels in both males and females and mineralization and necrosis in lower incidence also in both sexes incidences of chronic atrophic gastritis was high. Gastric tumours were found in two high dose males. The incidence of hepatocellular adenomas, hepatocellular carcinomas and hepatoblastomas was high in both treated and control male and female mice. While control males and females showed tumour rates of 63% (31/49) and 50% (25/50), respectively, and all treatment groups of males and females had tumour rates in excess of 92% with the exception of high dose male rates in which the tumour rate was 82% (41/50). Evidence of infection by <i>H. hepaticus</i> was found by PCR-RFLP, but associated hepatitis was not found.</p>

Conclusion Remarks	The authors determined that under the conditions of these 2-year gavage studies there was evidence of carcinogenic activity of methyl eugenol for male or female B6C3F1 mice at the dose levels tested.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (2000) Toxicology and carcinogenesis studies of methyl eugenol in F344/N Rats and B6C3F1 mice. NTP-TR-491. U.S. Dept of Health and Human Services. NIH Publication No. 98-3950.

4.4 Reproductive Toxicity

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol. Members of this class of substance including estragole are known to metabolize via a 1'-hydroxylation pathway to yield a reactive hepatotoxic metabolite.
Test Type	One generation
GLP	No
Year	1973
Species/Strain	Mouse/CD-1 outbred
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Days 6 to 15 of gestation
Doses/Concentration	0(control), 6, 26, 120, 560 mg/kg bw/day and a positive control of 150 mg/kg bw/day of aspirin.
Premating Exposure period for males	None
Premating Exposure period for females	None
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml/kg); Positive control received 150 mg/kg bw/day of aspirin in corn oil.
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, virgin adult female CD-1 outbred mice were gang-

housed in plastic disposable cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, females were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 150 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.

NOAEL(NOEL)

>560 mg/kg bw/day (equivalent to approximately 112 mg/kg/d of allylalkoxybenzene derivatives)

Actual dose received by dose level and sex

>560 mg/kg bw/day

Parental data and F1 as appropriate

Data for number of females mated/pregnant at each dose level: 0 mg/kg bw, 24/21; 150 mg/kg bw of aspirin, 30/20; 6 mg/kg bw, 30/22; 26 mg/kg bw, 31/21; 120 mg/kg bw, 22/21; 560 mg/kg bw, 32/20. All pregnant females survived to sacrifice on Day 17. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 11, 15, or 17 of the study. None of the pregnant females died or aborted before Day 17 and all litters were alive on Day 17 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 12.5; 150 mg/kg bw aspirin, 12.0; 6 mg/kg bw, 12.3; 26 mg/kg bw, 11.2; 120 mg/kg bw, 12.9; 560 mg/kg bw, 11.2. The average number of implantation sites/dam and % partial resorptions were similar for all groups: 0 mg/kg bw, 11.8 and 19%; 150 mg/kg bw aspirin, 11.3 and 45%; 6 mg/kg bw, 12.5 and 45%; 26 mg/kg bw, 11.9 and 28%; 120 mg/kg bw, 10.5 and 28%; 560 mg/kg bw, 11.0 and 25%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, was no evidence of toxicity to dams.

Offspring toxicity F1 and F2

Based on gross examination of live pups, visceral examination and skeletal examination there were no signs of toxicity to offspring. The total number of live fetuses, average number of live fetuses per dam, sex ratio, number of dead fetuses, and average fetal weight were not different between control and treatment groups. Total number of live fetuses/dead

Conclusion remarks	The administration of up to and including 560 mg/kg bw/day of test article FDA 71-28 to pregnant mice on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol
Test Type	One generation
GLP	No
Year	1973
Species/Strain	Hamster/adult golden
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Days 6 to 10 of gestation
Doses/Concentration	0(control), 6, 28, 130, or 600 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin
Premating Exposure period for males	None
Premating Exposure period for females	None
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml/kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil.
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, groups (26-28/dose/group) of virgin adult female hamster were individually housed in mesh-bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated one to one with untreated adult males and the appearance of motile sperm in the vaginal sperm was considered day 0 of gestation.

	<p>Beginning on Day 6 and continuing daily through Day 10 of gestation, females were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 8, 10, and 14 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 14 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.</p>
NOAEL(NOEL)	>600 mg/kg bw/day (equivalent to approximately 120 mg/kg/d of allylalkoxybenzene derivatives)
Actual dose received by dose level and sex	>600 mg/kg bw/day
Parental data and F1 as appropriate	<p>Data for number of females mated/ pregnant at each dose level: 0 mg/kg bw, 27/21; 250 mg/kg bw of aspirin, 26/19; 6 mg/kg bw, 28/19; 28 mg/kg bw, 26/21; 130 mg/kg bw, 28/20; 600 mg/kg bw, 27/23. All pregnant females survived to sacrifice on Day 14. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 8, 10, or 14 of the study. One death each was reported in the two control groups and in the two highest dose groups before day 14. All litters were alive on Day 14 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 10.3; 250 mg/kg bw aspirin, 9.9; 6 mg/kg bw, 9.6; 28 mg/kg bw, 11.4; 130 mg/kg bw, 9.6; 600 mg/kg bw, 11.2. The average number of implantation sites/dam and % partial resorptions were similar for all groups: 0 mg/kg bw, 11.7 and 15%; 250 mg/kg bw aspirin, 11.3 and 39%; 6 mg/kg bw, 12.1 and 32%; 28 mg/kg bw, 11.9 and 38%; 130 mg/kg bw, 11.5 and 42%; 600 mg/kg bw, 12.1 and 23%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, was no evidence of toxicity to dams.</p>
Offspring toxicity F1 and F2	Based on gross examination of live pups, visceral examination, and skeletal examination there were no signs of toxicity to offspring in either the control or test groups. The total number of live fetuses, average number of live fetuses per dam, sex ratio, and average fetal weight were not different between control and treatment groups. A small number of dead fetuses
Conclusion remarks	The administration of up to and including 600 mg/kg bw/day of test article FDA 71-28 to pregnant golden hamsters on days 6 through 10 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of

abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.

Data Reliabilities Qualities

Reliability code 2. Reliable with restriction.

Remarks for Data Reliability

Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.

References

Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Test Type	One generation
GLP	No
Year	1973
Species/Strain	Rat/adult Wistar
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Days 6 to 14 of gestation
Doses/Concentration	0(control), 3, 12, 56, or 260 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin.
Premating Exposure period for males	None
Premating Exposure period for females	None
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml/kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil.
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, virgin adult female Wistar were individually housed in mesh-bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, females were given 0, 3, 12, 56, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 20 all dams were

	<p>subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.</p>
NOAEL(NOEL)	>260 mg/kg bw/day (equivalent to approximately 52 mg/kg/d of allylalkoxybenzene derivatives)
Actual dose received by dose level and sex	>260 mg/kg bw/day
Parental data and F1 as appropriate	<p>Data for number of females mated/ pregnant at each dose level: 0 mg/kg bw, 25/23; 250 mg/kg bw of aspirin, 25/22; 3 mg/kg bw, 25/25; 12 mg/kg bw, 25/23; 56 mg/kg bw, 25/22; 260 mg/kg bw, 25/21. All pregnant females survived to sacrifice on Day 20. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 11, 15, or 20 of the study. None of the pregnant females died or aborted before Day 20 and all litters were alive on Day 20 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 12.8; 250 mg/kg bw aspirin, 11.1; 3 mg/kg bw, 12.7; 12 mg/kg bw, 12.5; 56 mg/kg bw, 11.6; 260 mg/kg bw, 10.7. The average number of implantation sites/dam and % partial resorptions were similar for all groups: 0 mg/kg bw, 11.9 and 9%; 250 mg/kg bw aspirin, 11.1 and 32%; 3 mg/kg bw, 12 and 12%; 12 mg/kg bw, 11.8 and 4%; 56 mg/kg bw, 11.1 and 5%; 260 mg/kg bw, 11.1 and 5%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, there was no evidence of toxicity to dams.</p>
Offspring toxicity F1 and F2	Based on gross examination of live pups, visceral examination, and skeletal examination there were no signs of toxicity to offspring in either the control or test groups. The total number of live fetuses, average number of live fetuses per dam, sex ratio, and average fetal weight were not different between control and treatment groups. A small number of dead fetuses
Conclusion Remarks	The administration of up to and including 260 mg/kg bw/day of test article FDA 71-28 to pregnant Wistar rats on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpublished report.

4.5 Developmental/Teratogenicity Toxicity

Substance Name	Safrole (analog for estragole)
CAS No.	94-59-7
Remarks for Substance	Data is for surrogate chemical 3,4-dimethylenedioxyallylbenzene (safrole). Substance is known to form reactive 1'-hydroxy metabolite (Miller et al., 1983)
Test Type	Developmental toxicity
GLP	No
Year	1985
Species/strain	Mice/Swiss
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Days 6-14 of gestation
Doses/concentration Levels	0, 5, 50, 100, 150, or 200 mg/kg bw/day
Exposure Period	8 days
Frequency of Treatment	Daily
Control Group and Treatment	Olive oil vehicle
Remarks for Test Conditions	Groups of 15-25 female Swiss mice were given oral doses of safrole by gavage daily at 0, 5, 50, 100, 150, or 200 mg/kg bw/day in olive oil for 8 days from days 6 to 14 of gestation. Males were untreated. Pregnant females were sacrificed on day 18. Parameters monitored included survival of females, number pregnant on day 18, number of implantations, number and % reabsorbed, number of live fetuses, mean foetal weight, and number and % of malformations per dosed group. Malformations were further classified according to anomalies of the cranium, anterior and posterior phalangi, column vertebrate, and morphological irregularities and absence of sternbrae in untreated and treated groups. Foetal malformations of the palate, brain, limbs, and tail were also recorded.
NOAEL(NOEL) maternal toxicity	5 mg/kg bw/day
LOAEL(LOEL) maternal toxicity	50 mg/kg bw/day
NOAEL (NOEL)	5 mg/kg bw/day based on growth retardation in fetuses at

developmental toxicity	higher dose levels
LOAEL (LOEL) developmental toxicity	50 mg/kg/day
Actual dose received by dose level and sex	0, 5, 50, 100, 150, or 200 mg/kg bw/day
Maternal data with dose level	Maternal data indicated that survival among females was decreased in a dose dependent manner at dose levels of 100 mg/kg bw/day and above. The number pregnant was decreased at 100 (, 150 and 200 mg/kg bw/day but this paralleled survival rates. The number of implantations also decreased at dose levels of 100 mg/kg bw/day and above. Signs of maternal toxicity were recorded at dose levels equal to and equal to and greater than 50 mg/kg bw/day. The % reabsorptions increased at doses equal to and greater than 50 mg/kg bw/day.
Fetal Data with Dose Level	<p>The mean foetal weight decreased at 50 mg/kg bw/day and above. There was no significant difference between foetal weight and survival between the controls and the 5 mg/kg/day dosed group. Although there was a statistically significant ($p < 0.001$) in malformations at the 50 and 150 mg/kg bw/day dosed groups there was no dose response. The authors noted that although there were significant signs of toxicity to dams and foetuses, there was no significant increase in malformations in the treated groups when compared to the control group. % malformations: control 9.2%, 5 mg/kg bw/day, 13.2%; 50 mg/kg bw/day, 19.4%; 100 mg/kg bw/day, 15.2%; 150 mg/kg bw/day, 19.4%</p> <p>The only consistent anomalies observed in treated groups were a malformation to the anterior and posterior phalange, but there was no direct dose dependent change among treated groups.</p>
Appropriate statistical evaluations	Not given
Conclusion Results	Safrole did not cause any significant increase in malformations in mice foetuses at all administered dose levels. Maternal toxicity and foetal toxicity were noted at doses equal to and greater than 50 mg/kg bw/day.
Data Qualities Reliabilities	Reliability code 123. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Comparable to guideline study.
References	Moro M.G., Ognio E., Rossi L, Ferreri Santi L., and Santi L. (1985) Prenatal toxicity of safrole in laboratory animals. Rivista Tossicologia Sperimentale Clinica, 15(1-2), 91-97.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and

	methyl eugenol Note: all substance mentioned arer metabolized via 1'hydroxylation.
Test Type	Teratology study
GLP	No
Year	1973
Species/strain	Mouse/CD-1 outbred
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	10 days
Doses/concentration Levels	0(control), 6, 26, 120, 560 mg/kg bw/day and a positive control of 150 mg/kg bw/day of aspirin
Exposure Period	Days 6 to 15 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml/kg); Positive control received 150 mg/kg bw/day of aspirin in corn oil
Remarks for Test Conditions	<p>Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female CD-1 outbred mice were gang-housed in plastic disposable cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (20-22/group) of pregnant females were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 150 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary).</p>

NOAEL(NOEL) maternal toxicity	>560 mg/kg bw/day (approximately equal to a daily dose of 112 mg/kg bw for allylalkoxybenzene derivatives)
NOAEL (NOEL) developmental toxicity	>560 mg/kg bw/day
Actual dose received by dose level and sex	0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28)
Maternal data with dose level	Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female mice. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group.
Fetal Data with Dose Level	The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses were similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Skeletal examination of sternbrae showed no significant differences in the incidence of incomplete ossification or missing sternbrae for test and control groups. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure was similar for test and control animals. Visceral examination failed to reveal any evidence of abnormalities at any dose level.
Conclusion Results	There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 560 mg/kg bw/day of test material.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol
Test Type	Teratology study
GLP	No
Year	1973
Species/strain	Rat/female Wistar
Sex	Female
Route of Administration	Oral-Gavage

Duration of Test	10 days
Doses/concentration Levels	0(control), 3, 12, 56, 260 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin
Exposure Period	Days 6 to 15 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml/kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female rats were individually housed in mesh bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (21-25/group) of pregnant females were given 0, 6, 26, 120, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 20 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary).
NOAEL(NOEL) maternal toxicity	>260 mg/kg bw/day (approximately equal to a daily dose of 52 mg/kg bw for allylalkoxybenzene derivatives)
NOAEL (NOEL) developmental toxicity	>260 mg/kg bw/day
Actual dose received by dose level and sex	0, 3, 12, 56, or 260 mg/kg bw of the test material (FDA 71-28)
Maternal data with dose level	Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group.
Fetal Data with Dose Level	The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live

fetuses were similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Except for positive control group, skeletal examination of sternbrae showed no significant differences in the incidence of incomplete ossification or missing sternbrae for test and untreated control group. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure were similar for test and the untreated control group except for the positive aspirin-treated control group in which increases in incidences of these skeletal effects were observed. Visceral examination failed to reveal any evidence of abnormalities at any dose level.

Conclusion Results	There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 260 mg/kg bw/day of test material.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol
Test Type	Teratology study
GLP	No
Year	1973
Species/strain	Hamster/female golden
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	5 days
Doses/concentration Levels	0(control), 6, 28, 130, 600 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin
Exposure Period	Days 6 to 10 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml/kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female

hamsters were individually housed in mesh bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated one to one with untreated young adult males and the appearance of motile sperm in the vaginal sperm was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, groups (19-23/group) of pregnant females were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 8, 10, and 14 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 14 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary).

NOAEL(NOEL) maternal toxicity	>600 mg/kg bw/day (approximately equal to a daily dose of 120 mg/kg bw for allylalkoxybenzene derivatives)
NOAEL (NOEL) developmental toxicity	>600 mg/kg bw/day
Actual dose received by dose level and sex	0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28)
Maternal data with dose level	Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group.
Fetal Data with Dose Level	The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses were similar for test and control groups. A small % of (less than 3%) dead fetuses were observed at the three highest dose levels. Skeletal examination of sternbrae showed no significant differences in the incidence of incomplete ossification or missing sternbrae for test and control groups. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closures were similar for test and control animals. Visceral examination failed to reveal any evidence of abnormalities at any dose level.
Conclusion Results	There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 600 mg/kg bw/day of

test material.

Data Qualities Reliabilities

Reliability code 2. Reliable with restriction.

Remarks for Data Reliability

Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.

References

Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpublished report.